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TRANSFER OF NEMERTEAN EGG PREDATORS DURING HOST MOLTING AND COPULATION

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Abstract

Juvenile nemertean egg predators were able to efficiently transfer from the premolt cuticle to the postmolt cuticle of male and female crabs when the host molted. These worms also efficiently transferred from male to female hosts at copulation. The synchronized responses of the nemertean worms to host physiology and behavior dramatically concentrate the nemertean population on the sole food source required for worm reproduction: crab eggs. The efficient location of reproductive crabs by juvenile worms increased the likelihood that these worms can have significant effects on crab fisheries when worm population density is high.

Introduction

Worms of the nemertean genus *Carcinonemertes* are ectosymbionts that feed on the eggs of decapod crustaceans. Infestation of hosts follows a planktonic larval period. The juvenile worms then ensheath in various protected spots on the host exoskeleton (Humes, 1942; Kuris, 1978; Wickham, 1980). These quiescent worms apparently subsist on dissolved organic matter leaked from crab arthrodial membranes and absorbed through the tegument of the worms (Roe *et al.,* 1981; Crowe *et al.,* 1982). *Carcinonemertes errans* infests the dungeness crab, *Cancer magister,* and will settle on crabs of any age or sex (Wickham, 1980). This crab is long lived and females produce their first egg clutch when two or three years old (Butler, 1961). Mating occurs only at the female molt. Males sequester premolt females and copulation occurs immediately following ecdysis of the female (Snow and Nielson, 1966). They reproduce once a year, brood eggs for about 90 days in central California, and must molt before producing subsequent broods. Worms infesting this crab species must feed on crab eggs in order to reproduce themselves.

In 1974 the average burden of nemerteans on ovigerous female dungeness crabs in central California was 29,000 *C. errans* per crab causing the mortality of over 50% of the crab eggs produced in the San Francisco region (Wickham, 1979). This level of infestation still persists and has shown no evidence of decline even though host abundance has been dramatically reduced relative to historical levels for this entire time period. Nemertean abundance on dungeness crabs from northern California, Oregon, and Washington has increased from 1000–2000 in 1974 and 1975 to levels comparable to those found in central California with a concomitant increase in crab egg mortality, possibly in response to record high populations of host crabs followed by a drastic cyclic decline (Wickham, 1980; unpubl.)

Nemerteans have been observed on many species of decapod crustaceans, occasionally with large numbers of worms on individual hosts (Humes, 1942; Aiken *et*

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al., 1983) but never to the extent found on *Cancer magister.* The only other instance of widespread infestations in epidemic proportions by nemerteans is on the Alaskan king crab, *Paralithodes camtschatica.* Populations over much of the range of this crab are currently suffering dramatic brood wastage apparently related to a marked increase in the abundance of nemerteans which are either new to this host, or, more likely were not observed previously on this heavily studied host because of typically low numbers (Wickham and Kuris, unpubl.) Factors which allow the maintenance of such large densities of ectosymbionts on hosts which periodically molt and have been thought to shed their symbionts at the molt (Humes, 1942) must be identified to understand the role of these organisms in their hosts' dynamics.

Materials and Methods

Specimens of juvenile and mature *Cancer magister* with resident populations of *Carcinonemertes errans* were collected from the waters near Bodega Bay, California. Mature male and female crabs which showed evidence of approaching molt were held separately in laboratory tanks and monitored through the molt. Worm distributions over the host exoskeleton were assayed by enumerating worms at a representative selection of sites normally occupied by worms. These included walking legs, axillae, chelipeds, thorax, abdomen, pleopods (female), or copulatory appendages (male). Photographs were taken to display worm disposition at the time of ecdysis. Worms were counted on the exuviae after molt. Redistribution of the worms on the new exoskeleton was followed over the first few days following molting by sampling the original sites. An exhaustive census of worms on the new exoskeleton was then conducted to determine the proportion of the original population which transferred at molt.

Intermolt transfer was also observed on juvenile crabs held in cubicles in plastic parts boxes. These crabs had low numbers of worms and, in 7 of 12 instances, worm populations on them were augmented by the addition of worms to the exoskeleton prior to ecdysis. The additional worms were obtained from adult male crabs. Worms crawled onto juvenile crabs placed with the worms in finger bowls. Observations of worm behavior at host ecdysis were conducted only after an acclimatization period of approximately a week.

Transfer of worms to females at mating was observed during two mating instances in two fashions. In the first instance the exoskeleton of a female was cleaned by sparging with 95% ethanol until all worms were killed and removed. She was then mated with an infested male and the number of worms on her exoskeleton was counted the following day and compared with the number remaining on the male to determine the proportion transferred. In the second instance an infested female was mated with a male whose worms had been vitally stained with neutral red dye. The distribution of these stained worms was then noted on the female the day after mating and the number was again compared with the number remaining on the male.

Observations were also made with *C. epialti* worms on the crab *Hemigrapsus oregonensis.* The proportion of worms transferring at molt was measured in the same fashion as with *C. magister.*

Results

When mature female *Cancer magister* molt, an average of 88% of the resident worms migrate to the new exoskeleton (Table I). The manner of migration is active, directed and strikingly illustrated in Figure 1. Several days prior to host ecdysis worms become more concentrated near the base of the abdomen where they have greater

TABLE I

Numbers of Carcinonemertes errans *on adult female crabs and exuviae after molting*

Crab No.	No. worms on exuvium	No. worms on new crab	Total no. worms	Proportion of worms transferring
1	134	1891	2025	.934
2	277	899	1166	.762
3	89	1612	1701	.948
4	827	5536	6363	.870
				$\bar{x}$ = .88
				S.D. = .09

access to the epimeral suture. Worms crawl toward the epimeral suture when decalcification begins. By the time the suture opens most of the worms are massed against the new exoskeleton of the crab so they can remain with the crab as the exuvium is shed.

Shortly after ecdysis worms can be observed migrating forward and outward until they assume a typical distribution pattern (see Table IV). During intermolt periods worms tend to be contracted and clustered in groups. On the early post-ecdysial crab, worms are elongated and can often be seen moving across calcified portions of the exoskeleton where they normally are not found. At this time worms can often be found head to tail in a single file along suture lines in the axils.

Transfer at ecdysis on male crabs appears to be more variable than on females.

FIGURE 1. Worms actively crawling toward decalcifying epimeral suture on postero-lateral surface of carapace.

TABLE II

Numbers of Carcinonemertes errans *on adult male crabs and exuviae after molting*

	Crab no.	No. worms on new crab	No. worms on exuvium	Total no. worms	Proportion of worms transferring
Spring	1	22	3	25	.12
	2	14	5	19	.26
	3	24	3	11	.27
	4	145	27	145	.16
	5	124	22	146	.15
					$\bar{x}$ = .19
					S.D. = .06
Late summer	1	25	377	402	.94

During spring when male crabs carried lower worm burdens most worms were lost with the molt. Worms on the male crab which molted in late summer transferred with an efficiency comparable to those on female crabs (Table II).

On juvenile crabs worms transferred on both sexes (Table III). Worms disappeared from the system in these experiments. An average of only 22% of the premolt worms were found on both the post molt and exuviae with male crabs (n = 6) and 73% of the premolt worms found with female (n = 9) post molt and exuviae. Comparable figures for loss to the system on adult crabs are not available given the difficulty of quantifying total worm abundance on pre-molt mature female crabs without first sacrificing the crab.

During the two mating instances approximately 90% and 85% respectively of the worms from the male transferred to the female (400 of approximately 5000, and 600 of approximately 3200 worms were left on the males after mating). In the observations done on worms dyed with neutral red the worms from males were distributed in a different pattern than the resident worms (G-test, $P < .01$). They tended to be slightly more concentrated under the abdomen (thoracic sutures plus bases of pleopods) than the preexisting population of worms on the female (Table IV).

The typical distribution of juvenile worms on intermolt males was much more concentrated in sites under the abdominal flap than on intermolt females (Table V).

Worms on the host *Hemigrapsus oregonensis* transferred in a fashion similar to that found with worms on *Cancer magister* but with somewhat less efficiency. Worms transferred more efficiently on male crabs (Table VI).

DISCUSSION

The striking behavior exhibited by *Carcinonemertes errans* on the crab *Cancer magister* at the molt represents an important adaptation for increasing the likelihood

TABLE III

Average number of Carcinonemertes errans *on premolt and postmolt juvenile* Cancer magister *and their exuviae (±S.D.)*

	Crab sex	Average no. worms on exuviae	Average no. worms on new crab	Average proportion transferring
M	(n = 7)	.57 (±.8)	7.43 (±4.8)	.93 (±.1)
F	(n = 5)	.60 (±1.3)	4.40 (±2.1)	.94 (±.1)

TABLE IV

Distribution of worms dyed with neutral red compared to undyed worms on adult female crab after mating

Site on crab	No. dyed worms	No. undyed worms	Proportion of total dyed	Proportion of total undyed
Chela	2	16	.01	.03
Coxal-basis joint on walking leg	35	101	.13	.17
Merus-carpus joint on walking leg	9	37	.03	.06
Thoracic sutures under abdomen	155	235	.59	.40
Bases of pleopod	60	192	.23	.33

of eventual arrival on a crab egg clutch where worms can complete their life cycle. The manner of movement by worms prior to actual ecdysis suggests that worms receive some type of preliminary information which causes them to begin to move toward areas with more direct access to the epimeral suture. The normal location of worms on males suggests that there is some recognition system involved in site selection. Juvenile worms on females can be found in virtually any protected crevice on the ventral surface of the body and on the limbs. On males most juvenile worms are under the folded abdomen near the copulatory appendages. This obviously facilitates transfer to females at mating but it also suggests that worms can distinguish male from female hosts and modify their site of infestation accordingly.

The nature of the signal for nemertean concentration near the abdomen just prior to ecdysis is unknown. Juvenile *C. errans* occur on or near arthrodial membranes which are known to be "leaky" (Crowe *et al.*, 1982). Worms actively absorb dissolved organic matter being leaked by the host at these sites (Roe *et al.*, 1981) so it is possible that alteration of the chemical nature of worm infestation sites provides a cue. One event which occurs at the time of molting is the separation of the epicuticle from the newly forming cuticle below, which could possibly alter the rate of leakage through

TABLE V

Distribution of juvenile worms on intermolt host exoskeletons (±S.D.)

	Male (n = 6)		Female (n = 3)	
Site	No. worms	Proportion of total worm pop.	No. worms	Proportion of total worm pop.
Axillae	24.0 (±13.7)	.06	167.3 (±16.7)	.12
Walking legs	10.3 (±4.4)	.02	891.3 (±276.3)	.61
Chelipeds	0.0	.00	6.7 (±5.8)	.01
Thorax	141.3 (±21.2)	.33	54.3 (±28.2)	.04
Abdomen	183.8 (±33.1)	.43	133.7 (±73.5)	.09
Pleopods or copulatory appendages	65.0 (±32.5)	.15	200.0 (±130.0)	.13
Proportion under abdomen	(.92) G test, $P < .01$		(.26)	

TABLE VI

Proportion of worms transferring to the new molt on male and female Hemingrapsus oregonensis *(±S.D.)*

Sex	No. worms on exuvium	No. worms on new crab	Average proportion transferring
M (n = 7)	0.14 (±.38)	1.71 (±.76)	.95 (±.12)
F (n = 12)	2.25 (±1.5)	3.00 (±4.2)	.46 (±.35)

the membranes or worms could sense molting fluid present between old and new cuticle.

The juvenile nemerteans on a pre-molt adult crab appear to aggregate where they have access to the epimeral suture but it is not until the suture actually begins to open that they migrate out across open calcified carapace to reach the suture. Again the chemicals which might be the actual attractants are unknown. Clearly the decalcification occurring there is a unique chemical event on the host and any number of compounds such as enzymes or breakdown products could be utilized as a cue for worm migration. The process of transfer on molting female crabs appears to be a multi-stage process involving recognition of diverse agents and specific action patterns. In contrast the shift from male to females at mating may be a one-stage process cued by the ecdysis of the female crab. Males mate with females just after the female has shed her exoskeleton and it is then that transfer from the male to female by worms occurs. In the transfer of worms marked with neutral red, worms from the male were more concentrated under the female abdomen relative to the distribution of the worms already on the female. It is not known if the male-derived worms would eventually have spread out similar to the worms already there.

The different behavior of worms on male crabs that molt before and after the spring mating season is consistent with our other observations and reinforces the adaptive behavior for *C. errans*. The one example of efficient transfer occurred on a male in late summer, several months after the mating season which is usually in February or March in central California. Worms on males transferring at this time become available for venereal transmission the next breeding season. Male crabs in early spring had a mean burden of 129.4 worms (n = 23). In contrast in the fall male crabs carried 1603.5 worms each (n = 29). The lower burden just after the mating season is consistent with the observation of venereal transfer.

The inability of a high proportion of worms to transfer at ecdysis on male crabs that molt immediately after the mating season is intriguing. Perhaps most of the remaining worms represent remnant population that were either unresponsive to previous transfer stimuli or are located in sites relatively inaccessible to such stimuli.

In the experiment involving transferral of worms on juvenile *Cancer magister* a large proportion of the worms disappeared and were found on neither the new crab cuticle nor on the exuvium. Most of this loss to the system occurred on male juvenile hosts but these were the hosts which had worms placed on them because of low original worm burdens. Perhaps acclimatization to the new host was insufficient resulting in worm departure. Worm transfer at molt was highly efficient in the worms which stayed in the experimental chambers.

The ability to transfer and concentrate on mature female crabs is highly adaptive for *C. errans*. It is only after feeding on host eggs that the worm can complete its own life cycle (Wickham, 1980). This transfer ability coupled with the fact that *C. errans* appears to be able to survive on amino acids leaked by the host for an indefinite

period (Roe *et al.*, 1981) means that once a larval worm finds a host it has a relatively high probability of eventually feeding on crab eggs.

The fact that *Carcinonemertes epialti* on the host *Hemigrapsus oregonensis* also is able to transfer at host ecdysis suggests that this behavior is general for *Carcinonemertes.* One other worm has been observed to transfer at host ecdysis on the host *Pinnixia tubicula* (J. McDermott, pers. comm.). Humes (1942) noted that *C. carcinophila* was shed with the exuvium when the host *Callinectes sapidus* molted. The location of worms on non-ovigerous hosts in the Atlantic differs in one important respect, however. *Carcinonemertes carcinonphila* is generally found sheathed between the gill lamellae of its host when not on an ovigerous crab (Humes, 1942; Hopkins, 1947). *Pseudocarcinonemertes homari* also lives in the branchial chamber and on the gills of the lobster *Homarus americanus* (Fleming and Gibson, 1981). On the Pacific coast, nemerteans have never been found in any number on the host gills (Wickham, 1978; unpubl.). It is possible that the location of the juvenile worms prevented transfer at molt, but in the case of the host *Pinnixa tubicola* the worms which transferred also lived in the host branchial chamber (J. McDermott, pers. comm.). Our observations of transfer by *C. errans* occurred because of the unusually high densities of worms involved. This behavior only became obvious when vast numbers of worms were observable. Further studies on transfer at molt by *Carcinonemertes carcinophila* are warranted.

The most significant consequence of these findings is *C. errans* has a heretofore unsuspected ability to accumulate on ovigerous crabs. This worm has been an enigma in that in central California crabs can carry as many as 100,000 worms. Yet *C. errans* has a fecundity on the order of 1000 eggs per year (Wickham, 1980) which is quite low when compared to other planktonically dispersed marine organisms. Efficiency in finding and remaining with hosts is critical to their success.

Thus, the predatory impact of *C. errans* on crab reproduction is maximized. Since the commercial season (for male dungeness crabs only) follows the channelization of the worm population to the reproductive female crabs, only a very small fraction of the worm population is lost to the principal source of crab mortality: the fishery. While crab populations may be temporarily depressed due to fishing, the worm population is buffered by the relative longevity of their unfished female hosts. The long-term consequences of the complex but efficient life cycle of *C. errans* for the dungeness crab fishery must now be explored.

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